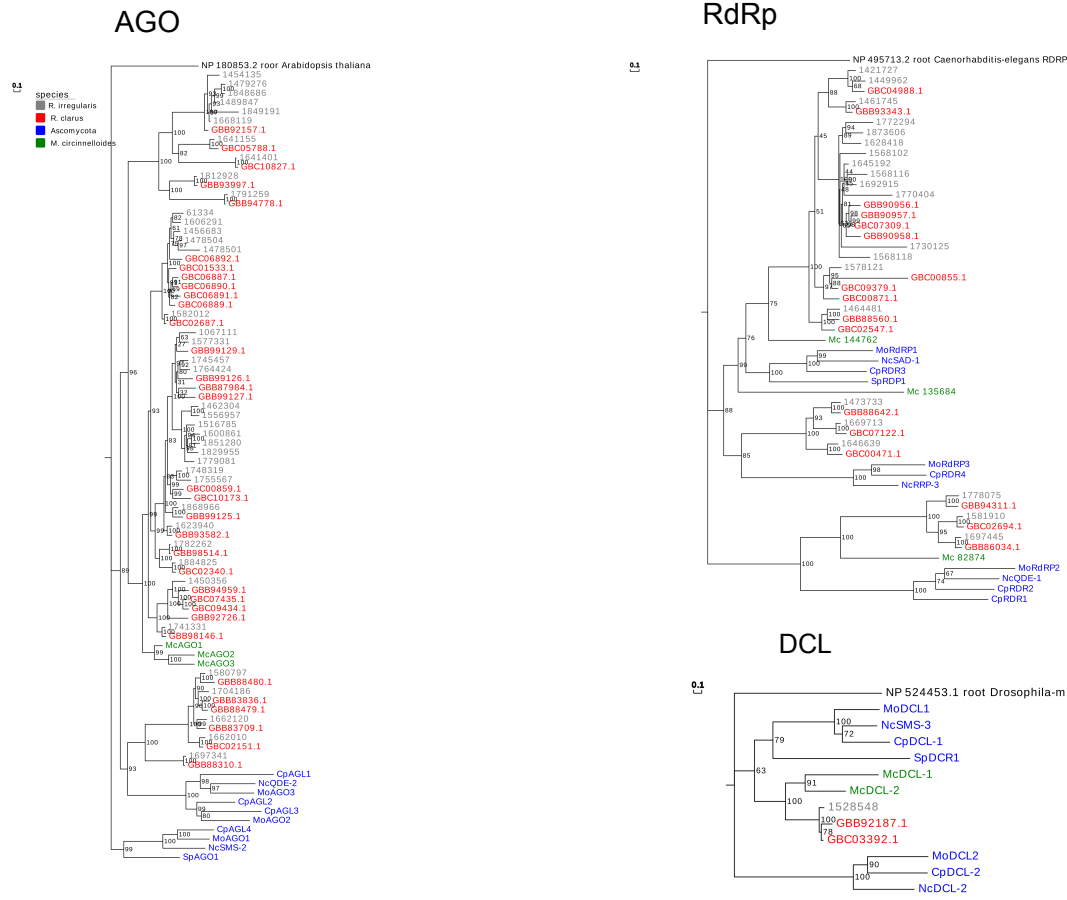
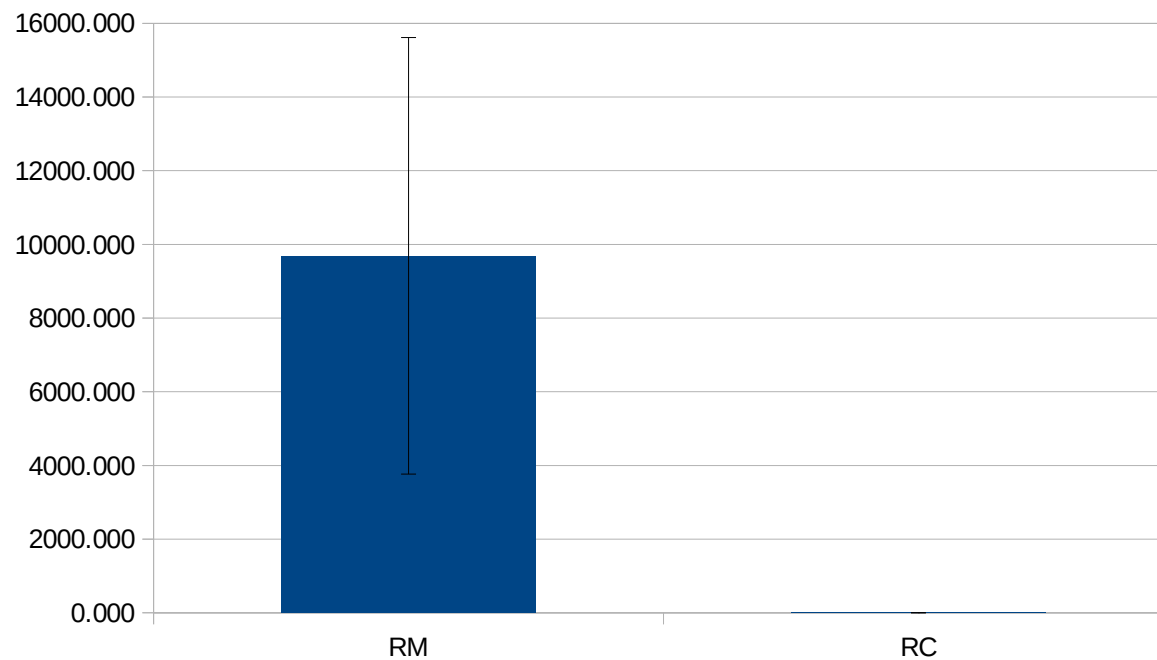


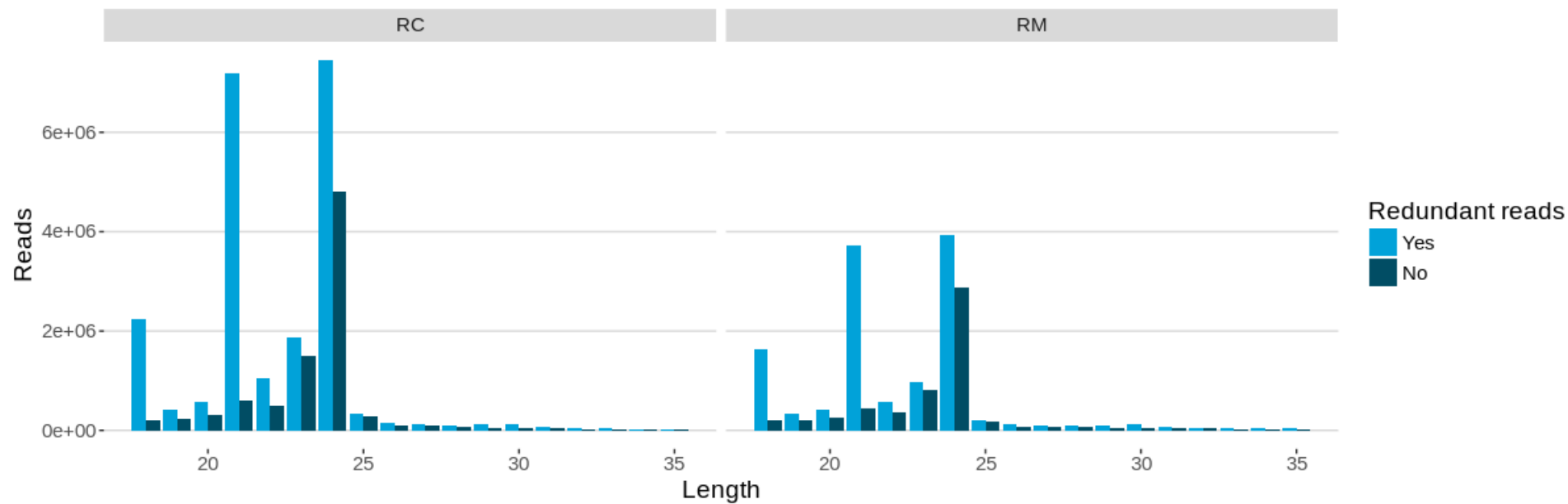
**Figure S2.** Phylogenetic relationship of RdRp proteins in different organisms. Proteins are discernible by species according to color and a two-letter prefix: Mo = *Magnaporthe oryzae*, Nc = *Neurospora crassa*, Mc = *Mucor circinelloides*, Sp = *Schizosaccharomyces pombe*, Cp = *Cryphonectria parasitica*, At = *Arabidopsis thaliana*. *Rhizophagus irregularis* proteins are identified by JGI numeric codes. *A. thaliana* proteins are identified (after the two-letter prefix) by FunRNA (Choi et al. 2014) ID. Protein ID of other species (NCBI or JGI): MoRdRP1= XP\_003721007.1, MoRdRP2 = XP\_003711624.1, MoRdRP3 = XP\_003712093.1, NcQDE-1 = EAA29811.1, NcSAD-1 = XP\_964248.3, NcRRP-3 = XP\_963405.1, SpRDP1 = NP\_001342838.1, McRdRP-1 = 111871, McRdRP-2 = 104159, CpRDR1 = 270014, CpRDR2 = 35624, CpRDR3 = 10929, CpRDR4 = 339656. The numbers at the nodes are bootstrap values (%) for 1000 replications. Tree was rooted using *Arabidopsis thaliana* Argonaute 6 (NCBI Reference Sequence: NP\_180853.2). Figure was generated with Evolview v2.



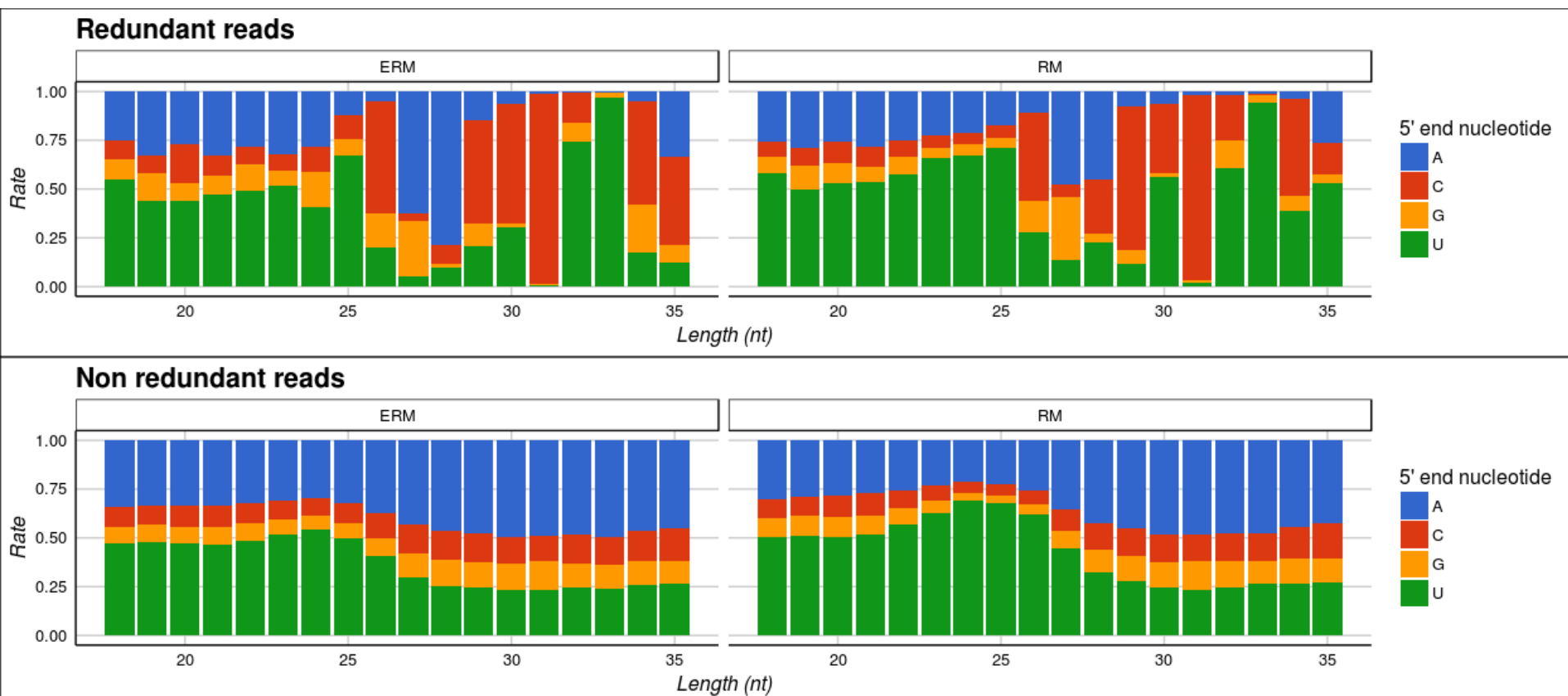
**Figure S3.** Phylogenetic relationship of AGO, RdRp and DCL proteins in different fungi. Proteins are discernible by species according to color and a two-letter prefix: Mo = *Magnaporthe oryzae*, Nc = *Neurospora crassa*, Mc = *Mucor circinelloides*, Sp = *Schizosaccharomyces pombe*, Cp = *Cryphonectria parasitica*. *Rhizophagus irregularis* proteins are identified by JGI numeric codes. *Rhizophagus clarus* proteins are identified with NCBI protein ID. Protein ID of other species (NCBI or JGI): MoAGO1 = XP\_003716704.1, MoAGO2 = XP\_003717504.1, MoAGO3 = XP\_003714217.1, NcQDE-2 = XP\_011394903.1, NcSMS-2 = EAA29350.1, SpAGO1 = O74957.1, McAGO-1 = 104161, McAGO-2 = 195366, McAGO-3 = 104163, CpAGL1 = ACY36939.1, CpAGL2 = ACY36940.1, CpAGL3 = ACY36941.1, CpAGL4 = ACY36942.1, MoRdRP1 = XP\_003721007.1, MoRdRP2 = XP\_003711624.1, MoRdRP3 = XP\_003712093.1, NcQDE-1 = EAA29811.1, NcSAD-1 = XP\_964248.3, NcRRP-3 = XP\_963405.1, SpRDP1 = NP\_001342838.1, McRdRP-1 = 111871, McRdRP-2 = 104159, CpRDR1 = 270014, CpRDR2 = 35624, CpRDR3 = 10929, CpRDR4 = 339656, MoMDL1 = XP\_003714515.1, MoMDL2 = XP\_003715365.1, NcSMS-3 = XP\_961898.1, NcDCL-2 = XP\_963538.3, SpDCR1 = NP\_588215.2, McDCL-1 = CAK32533.1, McDCL-2 = CAZ65730.1, CpDCL-1 = ABB00356.1, CpDCL-2 = ABB00357.1. Trees were rooted using: *Arabidopsis thaliana* Argonaute 6 (NCBI Reference Sequence: NP\_180853.2) for AGO, *Caenorhabditis elegans* RdRp (NCBI Reference Sequence: NP\_495713.2) for RdRp and *Drosophila melanogaster* Dicer 1 (NCBI Reference Sequence: NP\_524453.1) for DCL. The numbers at the nodes are bootstrap values (%) for 1000 replications. Figure was generated with Evolview v2.



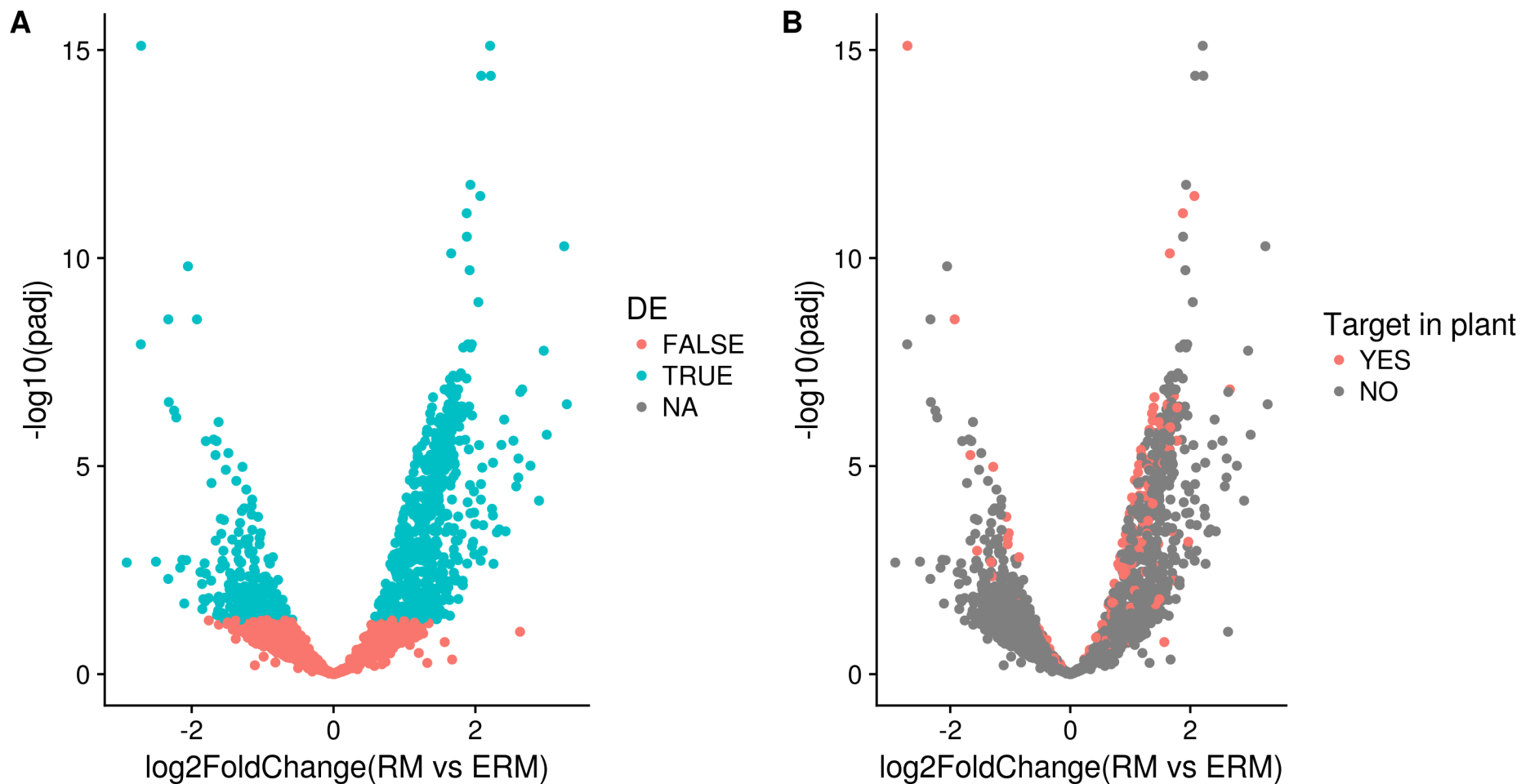
**Figure S4.** Expression of *MtPT4* relative to *MtTEF* assessed by qRT-PCR in RM samples (mycorrhizal roots) compared to RC ones (non-mycorrhizal roots). Data for each condition are presented as mean  $\pm$  standard error.



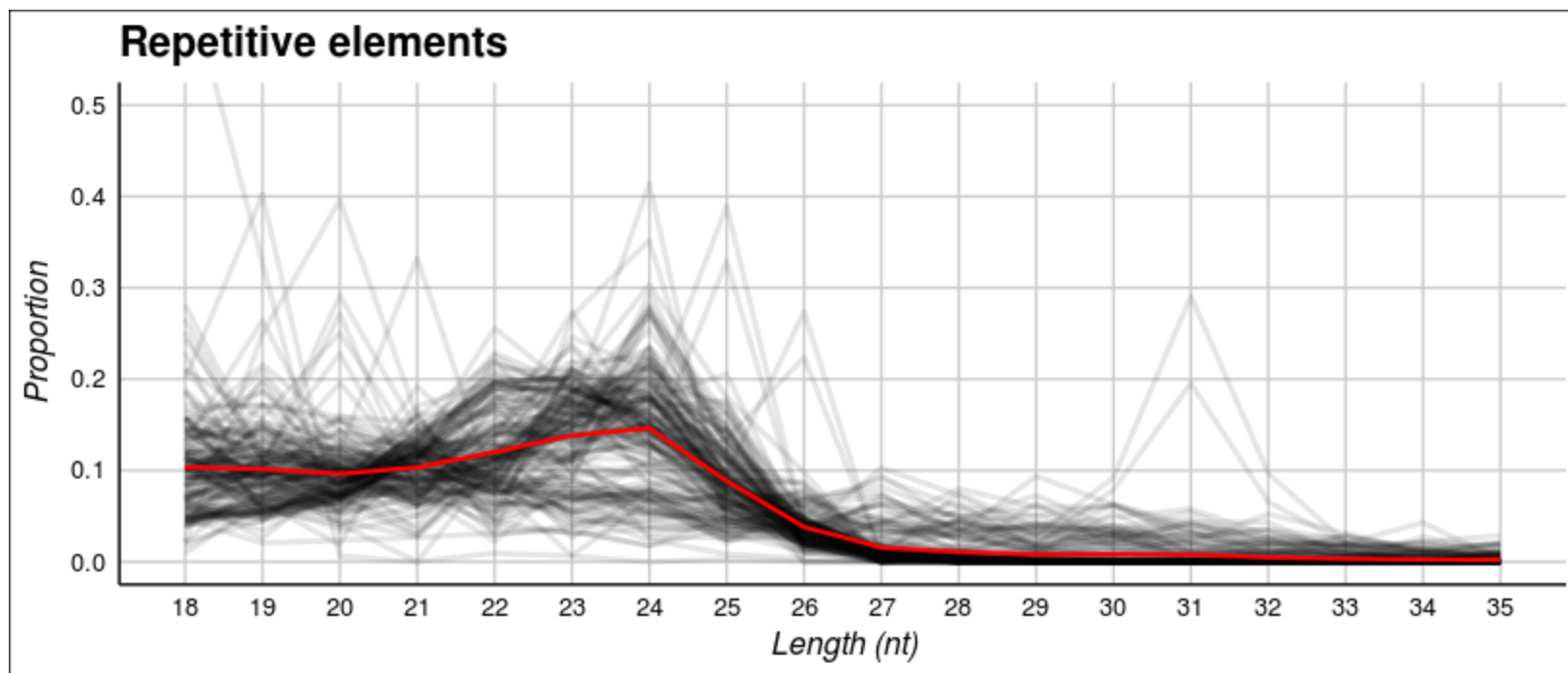
**Figure S5.** Length distribution (expressed in nucleotide) of sRNAs reads (redundant and non-redundant) from RC (non mycorrhizal roots) and RM (mycorrhizal roots) libraries mapping on *Medicago truncatula* genome.



**Figure S6.** Relative nucleotide frequency of 5' end of sRNAs reads (redundant and non-redundant) from RM (mycorrhizal roots) and ERM (extra radical mycelium) libraries mapping on *Rhizophagus irregularis* genome.



**Figure S7.** Volcano plots (fold changes vs adjusted p-values) of *Rir*-sRNA-generating loci. **A)** Blue dots (TRUE) represent the loci differentially expressed (adjusted p-values < 0.05) between ERM (extra radical mycelium) and RM (mycorrhizal root) conditions according to DESeq2 analysis (DE = differentially expressed); **B)** Red dots (YES) represent the loci that produce *Rir*-sRNAs targeting *Mtr*-mRNAs, according to sPARTA analysis.



**Figure S8.** Length distribution (in nucleotide) of sRNA reads that defined the *Rir*-sRNAs-generating loci homologous to repetitive elements in RepBase. Black lines are the length distribution of the individual loci and red line is the average length distribution of the plotted loci.